

WHAT IS CLAIMED IS:

1. A monoclonal antibody produced by a hybridoma formed by fusion of a myeloma cell and a ^{cell capable} ~~cell capable~~ of producing antibody against a prostate epithelium specific antigen, which monoclonal antibody ^{binds specifically to} ~~is reactive with~~ an epitope present on a membrane associated, ~~non-secretory~~ antigen of human prostatic cancer epithelium and normal prostatic epithelium.

2. The monoclonal antibody of claim 1, which further is capable of binding to and immunologically ^{staining} ~~staining~~ prostatic epithelium.

3. The monoclonal antibody of claim 1, which intensely stains prostatic cancer cells on the periphery of said cells with a small degree of heterogeneity and weakly stains normal prostatic cells and nonmalignant prostatic ductal epithelium.

4. The monoclonal antibody of claim 3, which is further capable of differentiating prostatic carcinomas from a) ^{non-prostatic cancers} ~~other carcinomas~~ and b) normal tissue of bladder, urethra, testis, ovarian, uterus, colonic, breast, thyroid, pancreatic or skin histotypes.

5. The monoclonal antibody of claim 1 or 2 which is of class IgG.

6. The monoclonal antibody of claim 1 or 2 which is of subclass IgG1.

7. The monoclonal antibody of claim 1 or 2 wherein the cell capable of producing antibody against a prostate epithelium specific antigen is (a) derived from an animal immunized with a metastatic lesion of human prostatic carcinoma and (b) is selected from the group consisting of spleen cells, lymph node cells, and peripheral blood lymphocytes.

8. The monoclonal antibody of claim 7, wherein the human prostatic carcinoma cells ^{originating} ~~are derived~~ from cells expressing organ or tissue specific prostate antigens.

claim 1

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9. The monoclonal antibody of claim ~~8~~^{10 or 2}, wherein the human prostatic carcinoma cells are derived from cell line LNCaP.

5 a a 10. The monoclonal antibody of claim ~~1, 7 or 8~~^{10 or 2}, wherein the myeloma cell is a mouse myeloma cell.

11. The ~~monoclonal~~^{hybridoma} antibody of claim ~~1, 7 or 8~~^{10 or 2}, wherein the myeloma cell is P3x63Ag8.653.

10 12. A monoclonal antibody of class IgG produced by a hybridoma formed by fusion of a P3x63Ag8.653 myeloma cell and a splenocyte capable of producing antibody against LNCaP prostatic carcinoma cells, which monoclonal antibody:

- (a) reacts specifically with human prostatic cancer cells and normal prostatic cells;
- 15 (b) differentiates prostatic carcinoma cells from other carcinoma cell types; ✓
- (c) does not react with normal tissues derived from bladder, urethra, testis, ovarian, colonic, breast, thyroid pancreatic or skin histotypes; and
- (d) reacts with a membrane associated non-secretory antigen of human prostate cells or tissue.

20 13. The monoclonal antibody of claim ~~12~~^{10 or 2}, produced by a hybridoma formed by fusion of a P3x63 Ag8.653 myeloma cell and a lymphocyte from a (Balb/c) mouse previously immunized with live LNCaP prostate cells.

25 a 14. The monoclonal antibody of claim ~~12~~^{10 or 2}, produced by a hybridoma formed by fusion of a P3x63Ag8.653 myeloma cell and a lymphocyte from a Balb/c mouse previously immunized with a (membrane) ~~isolated~~^{fraction} from human prostatic cancer cells.

30 15. The monoclonal antibody of claim ~~12~~^{10 or 2}, wherein the lymphocyte capable of producing antibody against LNCaP human prostatic carcinoma cells is derived from an individual with prostatic carcinoma.

170 31 16. A process for producing monoclonal antibodies from hybridoma cultures which comprises: ✓

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- (1) providing lymphocytes of mice previously immunized with plasma membrane enriched fractions of LNCaP cells or whole LNCaP cells;
- (2) fusing the lymphocytes with myeloma cells to form a hybrid cell line;
- (3) culturing the cell line in an in vitro culture medium to produce monoclonal antibodies; and
- 10 (4) harvesting from said in vitro cultures monoclonal antibodies which:
- (a) react specifically with human prostatic cancer cells and normal prostatic cells;
- (b) differentiate prostatic carcinoma cells from other carcinoma cell types;
- 15 (c) does not react with normal tissues derived from bladder, urethra, testis, ovarian, colonic, breast, thyroid, pancreatic or skin histotypes; and
- 20 (d) react with a membrane associated ~~non-secretory~~ antigen of human prostate cells or tissue.
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17. The process according to claim 16, wherein said myeloma cell is P3x63Ag8.653.

- 25 18. A process for producing monoclonal antibodies from hybridoma cultures which comprises:

- (1) taking lymphocytes from mice previously immunized with plasma membrane-enriched fractions of LNCaP cell;
- (2) fusing the splenocytes with a myeloma cell;
- 30 (3) culturing the cell line in vivo; and
- (4) harvesting from ascites fluid or sera of mice injected with said hybridoma monoclonal antibodies which:
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- (a) react specifically with human prostatic cancer cells and normal prostatic cells;
- (b) differentiates prostatic carcinoma cells from other carcinoma cell types;
- (c) does not react with normal tissues derived from bladder, urethra, testis, ovarian, colonic, breast, thyroid, pancreatic or skin histotypes; and
- (d) reacts with a membrane associated non-secretory antigen of human prostate cells or tissue.

19. The process according to claim 16 or 18, further comprising: screening of hybridoma cultures for production of monoclonal antibodies by immunoblotting, ELISA, ^{immunofluorescence} and immunoperoxidase staining.

20. A continuous cell line which produces a monoclonal antibody (which comprises:) a hybridoma of a lymphocyte derived from a mouse immunized with cells bearing a prostate specific antigen or an immunogenic determinant thereof derived from cells expressing organ or tissue specific prostate antigens and a mouse myeloma cell.

21. A continuous cell line which produces monoclonal antibodies which:

- (a) react specifically with human prostatic cancer cells and normal prostatic cells;
- (b) differentiates prostatic carcinoma cells from other carcinoma cell types;
- (c) does not react with normal tissues derived bladder, urethra, testis, ovarian, colonic, breast, thyroid, pancreatic or skin histotypes; and
- (d) reacts with a membrane associated non-secretory antigen of human prostate cells or tissue,

5 which is further characterized as a hybridoma of a lymphocyte derived from a mouse immunized with cells bearing a prostate specific antigen or immunogenic determinant thereof derived from cells expressing organ or tissue specific prostate antigens and a mouse myeloma cell.

10 22. A continuous cell line which produces a monoclonal antibody which comprises: a hybridoma of a lymphocyte derived from a mouse immunized with cells bearing a prostate specific antigen or an immunogenic determinant thereof derived from cell line LNCaP. ✓

15 23. A continuous cell line which produces monoclonal antibodies which:

- (a) react specifically with human prostatic cancer cells and normal prostatic cells;
- (b) differentiates prostatic carcinoma cells from other carcinoma cell types;
- (c) does not react with normal tissues derived from bladder, urethra, testis, ovarian, colonic, breast, thyroid, pancreatic or skin histotypes; and ✓
- (d) reacts with a membrane associated ~~non-secretory~~ antigen of human prostate cells or tissue,

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25 which is further characterized as a hybridoma of a lymphocyte derived from a mouse immunized with cells bearing a prostate specific antigen or immunogenic determinant thereof derived from cell line LNCaP.

cas 24. Hybridoma cell line 7E11-C5, ATCC No. HB *Designation HB 10494* ✓

25. Hybridoma cell line 9H10-A4, ATCC No. HB ✓

30 26. A monoclonal antibody 7E11 produced by the hybridoma cell line of claim 24.

27. A monoclonal antibody 9H10-A4 produced by the hybridoma cell line of claim 25. ✓

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28. A method for detecting prostate carcinoma, which comprises: contacting the monoclonal antibody of claim ~~1-7~~ ^{ok 2} ~~or 8~~ with a human tissue or fluid sample, and detecting an interaction of said antibody with any antigenically corresponding prostate carcinoma cells or antigenic determinants thereof in said sample.

29. The method according to claim 28, wherein the human tissue is prostate tissue.

30. The method according to claim 28, wherein the fluid sample is serum.

31. The method of claim 28, wherein the interaction is detected by immunohistological staining.

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32. A non-invasive competitive binding enzyme linked immunosorbent method for detecting prostate carcinomas in a human patient, comprising measuring the level of an antigen specific for prostate in a body tissue or fluid by:

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- (a) pre-incubating a sample containing an unknown amount of said antigen with a monoclonal antibody which is reactive with an epitope present in a membrane associated ~~non~~ secretory antigen of human prostatic cancer epithelium and normal prostatic epithelium;
 - (b) allowing sufficient time for the formation of monoclonal antibody-antigen complexes;
 - (c) removing the supernatant which did not react from step (a) and testing the supernatant for residual monoclonal antibody activity in an ELISA test comprising: ✓
 - (d) coating a surface of a solid phase with ~~—~~ derived from prostatic cells expressing organ or tissue specific prostate antigen and allowing the monoclonal antibody from step (c);

to react with said tissues for sufficient time to form monoclonal antibody-solid-phase tissue complexes;

(e) removing the monoclonal antibodies which did not react in step (d);

(f) reacting enzyme-conjugated antibodies with the monoclonal antibody-solid-phase tissue complexes of step (d) for sufficient time to form enzyme-conjugated antibody-monoclonal antibody-solid-phase tissue complexes;

(g) removing the enzyme-conjugated antibodies which did not react in step (f);

(h) contacting the enzyme-conjugated antibody-monoclonal antibody-solid-phase tissue complexes with a substrate of the conjugate enzyme, measuring enzyme activity, and quantitatively determining the percentage of inhibition of said antigen by comparing with a normal standard control sample.

33. The method according to claim 32, wherein the monoclonal antibody is produced by a hybridoma formed by fusion of a myeloma cell and a cell capable of producing antibody against a prostate specific antigen, which monoclonal antibody is reactive with an epitope present on a membrane associated, ~~non-secretory~~ antigen of human prostatic cancer epithelium and normal prostatic epithelium.

34. The method according to claim 32, wherein the cell capable of producing antibody against a prostate specific antigen is (a) derived from an animal immunized with a metastatic lesion of human prostatic carcinoma and (b) is selected from the group consisting of spleen cells, lymph node cells, and peripheral blood lymphocytes.

35. The method according to claim 33, wherein the human prostatic carcinoma cells are derived from cells expressing organ or tissue specific prostate antigens.

36. The method according to claim 32, wherein said fluid sample is sera.

37. The method according to claim 32, wherein said body tissue is prostate tissue.

38. The method according to claim 32, wherein the monoclonal antibody is MeAb 7E11-C4.

39. The method according to claim 32, wherein the tissue of step (d) are derived from LNCaP cells.

40. A method of passive immunotherapy for the treatment of prostate carcinoma, which comprises: (a) purifying the monoclonal antibody of claim 1, ^{or 2} ~~or 8~~; and (b) administering said monoclonal antibody to a prostate carcinoma patient in a suitable carrier.

41. A method of immunotherapy for the treatment of prostate carcinoma which comprises: (a) purifying the monoclonal antibody of claim 1, ^{or 2} ~~or 8~~; (b) conjugating said monoclonal antibody to a cytotoxic agent; and (c) administering said conjugated monoclonal antibody to a prostate carcinoma patient in a suitable carrier.

42. A method of immunotherapy for the treatment of prostate carcinoma which comprises: (a) purifying the monoclonal antibody of claim 1, ^{or 2} ~~or 8~~; (b) administering said monoclonal antibody to a prostate carcinoma patient, whereby anti-idiotypic antibody formation is elicited.